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13. ABSTRACT ***(Maximum 200 Words)***

Summary. The goal of this IDEA grant was to prepare a ^{18}F-labeled radiotracer having a high affinity and selectivity for the sigma-2 (σ_2) receptor that can be used for Positron Emission Computed Tomography (PET) imaging studies of breast cancer. Our choice of this receptor system stems from the observation that there is a high density of σ_2 receptors in both murine and human breast cancer cells in vitro. In addition, we have reported that the density of σ_2 receptors is about 10 times greater in proliferative mouse breast tumor cells than on quiescent mouse breast tumor cells in both cell cultures and in solid breast tumors growing in nude mice. These data suggest that a ^{18}F-labeled σ_2 radiotracer should have the potential to both image breast tumors and assess the proliferative status of these tumors in vivo with PET.

The goal of this IDEA grant was to prepare a ^{18}F-labeled ligand having a high affinity and selectivity for σ_2 versus σ_1 receptors based on our lead compound, **1** (Table I). The initial strategy involved a structure-activity relationship study aimed at exploring the nature of the substituent effects in the aromatic ring of the benzyl group of **1**. This study identified a number of compounds having a high affinity and selectivity for σ_2 versus σ_1 receptors. This initial study also identified compound **1b** as a potential ^{18}F-labeled radiotracer for imaging the σ_2 receptor status of breast tumors. In vivo biodistribution studies of [^{18}F]**1b** in tumor-bearing mice also revealed that a suitable tumor:background ratio was obtained for imaging purposes, but it required 4 hrs post-i.v. injection in order to reach this value. We are currently preparing compounds having a higher σ_2 receptor affinity than that of [^{18}F]**1b** with the goal of identifying a radiotracer that can produce a high tumor:background ratio in a shorter period of time following intravenous administration of the radiotracer, which should be better suited for imaging studies in breast cancer patients.able tumor:background ratio was obtained for imaging purposes, but it required 4 hrs post-i.v. injection in order to reach this value. We are currently preparing compounds having a higher σ_2 receptor affinity than that of [^{18}F]**1b** with the goal of identifying a radiotracer that can produce a high tumor:background ratio in a shorter period of time following intravenous administration of the radiotracer, which should be better suited for imaging studies in breast cancer patients.

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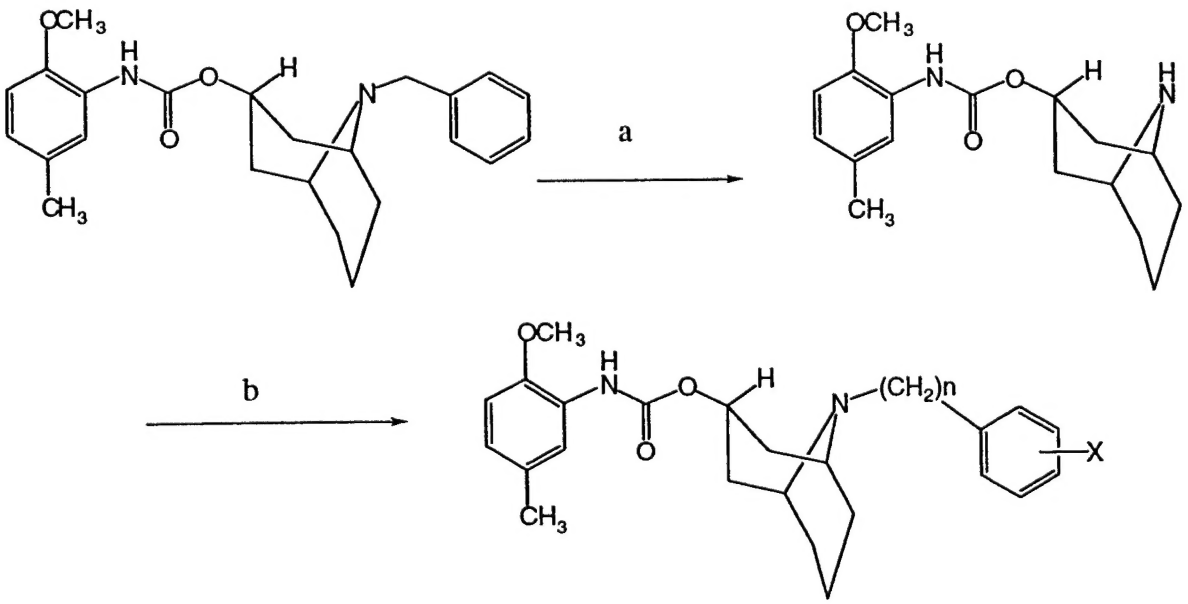
Introduction. The approach taken for the development of tumor imaging agents is the development of small molecule-based radiopharmaceuticals that have a high affinity for a receptor having an abnormal expression in tumor cells versus that of normal tissue. The research described in this application focuses on a ^{18}F-labeled small molecule that possesses a high affinity and selectivity for the σ_2 receptor. Our reasons for focusing on the sigma-2 (σ_2) receptor as the molecular target for tumor imaging was based on the following experimental observations:

- σ_2 receptors are expressed in high density in a number of solid tumors (Vilner et al., 1995).
- the density of this σ_2 receptors is higher in proliferative mouse mammary adenocarcinoma cells versus that of nonproliferative or quiescent mouse mammary adenocarcinoma cells (Mach et al., 1997).
- the upregulation and downregulation of this receptor follows the kinetics of mouse mammary adenocarcinoma cells to enter and exit the cell cycle. The kinetics of the up- and downregulation of the σ_2 receptor was found to correspond to that of PCNA, a known marker of proliferation (Al-Nabulsi et al., 1999).
- the 10-fold difference in σ_2 receptor density between proliferative and quiescent mouse mammary adenocarcinoma cells grown in cell culture also occurs in solid tumors of this cell line growing in nude mice (Wheeler et al., 2000).

The above experimental observations indicate that the σ_2 receptor is a potential molecular marker for imaging tumors. In addition, the studies from our laboratory indicate that the density of σ_2 receptors in proliferative breast tumors is 10-fold higher than that occurring in nonproliferative or quiescent tumors. Therefore, a σ_2-based imaging agent has the potential to provide information about the proliferative status of primary breast tumors.

Body. Previous studies from our lab have shown that the granatane analog, **1a**, displays a moderate affinity and modest selectivity for σ_2 versus σ_1 receptors (Mach et al., 2001). The goal of this research project is to improve the σ_2 binding affinity and σ_2: σ_1 selectivity ratio of the carbamate lead compound, **1** by exploring the nature of the substituent effect in the aromatic ring attached to the bridgehead nitrogen atom (X). The synthesis of the desired compounds is outlined in Scheme I. The results of this study revealed a number of potent and selective σ_2 ligands that are potential candidate ligands for PET radiotracer development (Table I).

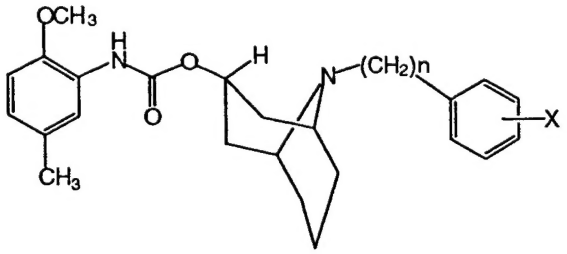
Scheme I



Reagents: a: H_2/Pd/charcoal/ethanol; b: benzyl iodide/Et_3N/DMF/90°C

The results of this initial study indicate that compound **1b** is a potential PET radiotracer for imaging the σ_2 receptor status of breast tumors. Furthermore, the corresponding F-18 labeled analog ([^{18}F]**1b**) was easily prepared via alkylation of the corresponding secondary amine with [^{18}F]4-fluorobenzyl iodide as outlined in Scheme I. Biodistribution studies were conducted with [^{18}F]**1b** in nude mice implanted with mouse mammary adenocarcinoma cells, line 66. The results of this study are shown in Table II and Figure 1. The results of this study indicate that there is a high uptake and suitable tumor:blood and tumor:muscle ratios for imaging purposes at 240 min post-i.v.-injection of the radiotracer. The goal of subsequent studies is to identify a ligand with a higher affinity for σ_2 receptors that can produce higher tumor:blood and tumor:muscle ratios at an earlier time point than that observed for [^{18}F]**1b**. Furthermore, co-injection with haloperidol, a known sigma ligand, was shown to reduce the tumor:blood ratio, which is consistent with the labeling of σ_2 receptors in vivo (Figure 2).

Table I. In vitro binding data for the N-substituted compounds for σ_1 and σ_2 receptors.

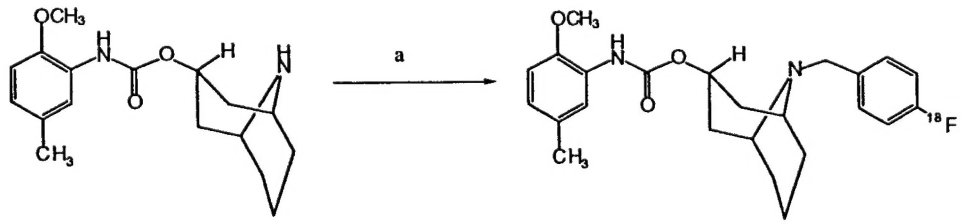


1a: n = 1, X = H
2a: n = 2, X = H

Compound	X	σ_1 [a]	σ_2 [b]	σ_1:σ_2 ratio[b]
1a	H	92.5 ± 11.0	3.1 ± 0.8	30
1b	4-F	202 ± 22	30.1 ± 2.0	6.7
1c	4-I	454 ± 78	30.6 ± 3.9	14.8
1d	4-NO_2	539 ± 42	25.2 ± 3.8	21.4
1e	4-CH_3	273 ± 22	15.4 ± 1.8	17.7
1f	2-F	>1,000	206 ± 13	>5
1g	3-F	322 ± 13	318 ± 51	1.0
1h	2-I	>1,000	>1,000	-
1i	3-I	>1,000	51 ± 4	>20
1j	2-NO_2	>1,000	>1,000	-
2a	H	59.9 ± 4.6	1.2 ± 0.1	50
2b	4-F	262 ± 37	5.9 ± 1.5	44
2c	4-I	175 ± 7	141 ± 8	1.3
2d	4-NH_2	2550 ± 72	5.0 ± 0.5	510
2e	4-NO_2	215 ± 39	11.5 ± 2.4	18.7
2f	4-CH_3	>1,000	8.6 ± 5.8	>100

[a]Ki for inhibiting the binding of [^{3}H](+)-pentazocine to guinea pig brain homogenates (n = 3); [b]Ki for inhibiting the binding of [^{3}H]DTG to rat liver homogenates (n = 3).

Scheme II



[a]Reagents: [^{18}F]4-fluorobenzyl iodide/Et_3N/DMF/90°C

Table II. Biodistribution study of [^{18}F]**1b**.

Organ	30 min	60 min	120 min	240 min
Brain	1.48 ± 0.19	1.92 ± 0.24	1.18 ± 0.19	0.13 ± 0.01
Blood	1.66 ± 0.10	3.07 ± 0.45	1.90 ± 0.18	0.37 ± 0.06
Lung	5.38 ± 0.85	5.11 ± 1.36	3.77 ± 0.39	0.65 ± 0.11
Heart	2.10 ± 0.18	3.30 ± 0.54	1.80 ± 0.21	0.31 ± 0.05
Liver	7.92 ± 1.18	10.93 ± 1.00	7.36 ± 0.90	1.14 ± 0.18
Kidney	6.40 ± 0.25	14.71 ± 4.57	5.50 ± 0.76	1.08 ± 0.11
Intestine	6.49 ± 0.69	17.92 ± 3.19	9.40 ± 0.87	2.23 ± 0.21
Muscle	1.25 ± 0.23	3.43 ± 0.58	2.72 ± 0.90	0.23 ± 0.02
Spleen	4.55 ± 0.33	5.87 ± 0.71	3.45 ± 0.39	0.55 ± 0.11
Tumor	1.34 ± 0.21	5.02 ± 0.85	3.18 ± 0.12	0.89 ± 0.03
Tumor: Blood Ratio	0.80 ± 0.08	1.66 ± 0.23	1.72 ± 0.13	2.48 ± 0.22
Tumor: Muscle Ratio	1.13 ± 0.27	1.78 ± 0.48	1.53 ± 0.29	3.98 ± 0.30

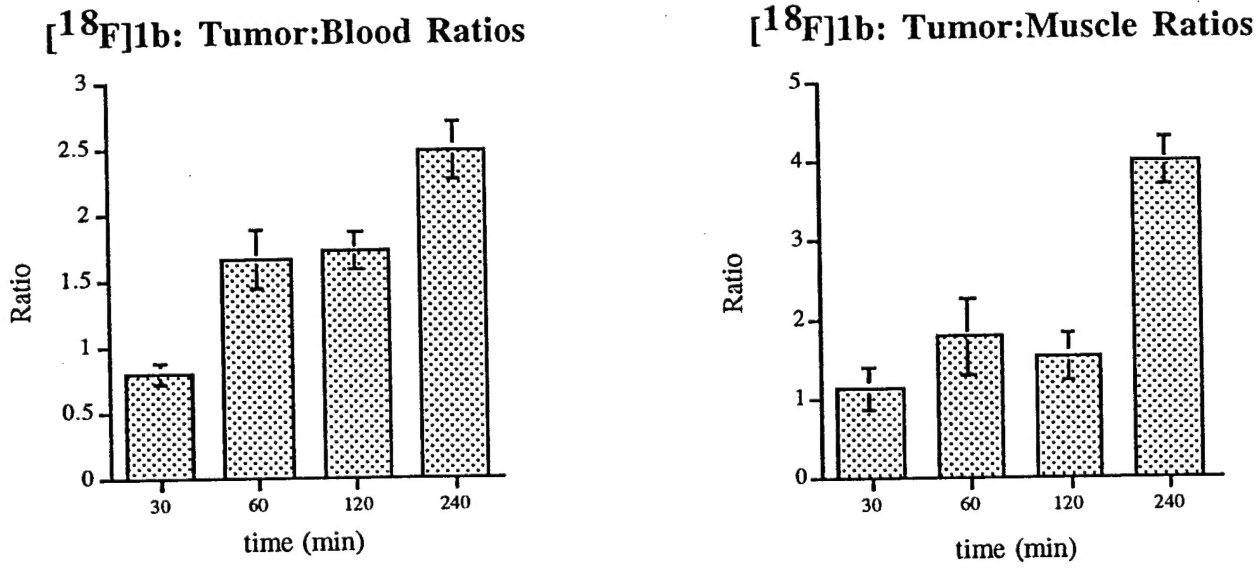


Figure 1. Tumor:blood and tumor:muscle ratios of [^{18}F]**1b** in tumor bearing mice.

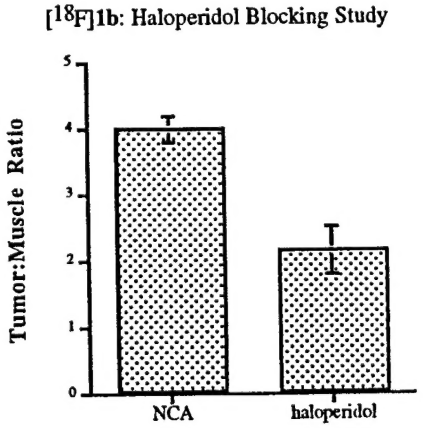
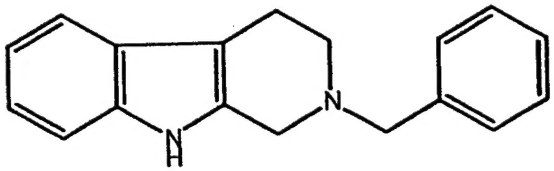
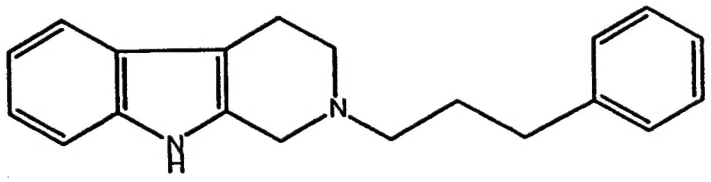


Figure 2. Haloperidol blocking study.

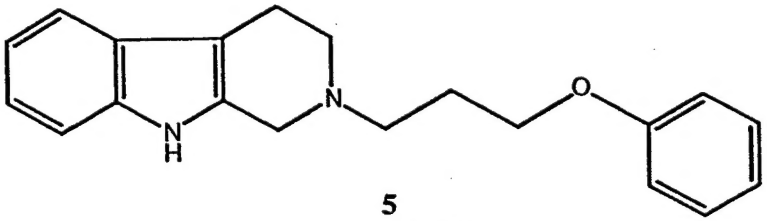
Rigid Analogs. We have also prepared a number of rigid analogs in which the conformationally-flexible carbamate moiety has been replaced 1,2,3,4-tertahydrocarboline ring system (Figure 3.) The carboline skeleton represents a rigid analog of **1** and **2** in which the aromatic ring of the phenyl carbamate moiety is fixed in a co-planar orientation to the piperidine ring. The structures and in vitro binding data of the first four compounds in this series are shown in Figure 3. These data suggest that it is possible to prepare σ_2-selective compounds using this strategy.



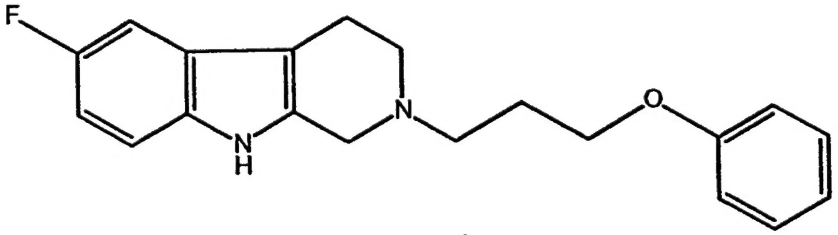
3
$\sigma_1 = 108$ nM
σ_2 >1,000 nM
σ_1: σ_2 ratio: 0.1



4
$\sigma_1 = 444$ nM
$\sigma_2 = 33.7$ nM
σ_1: σ_2 ratio: 13



5
$\sigma_1 = 614$ nM
$\sigma_2 = 37.4$ nM
σ_1: σ_2 ratio: 14



6
$\sigma_1 = 66.5$ nM
$\sigma_2 = 110$ nM
σ_1: σ_2 ratio: 0.6

Figure 3. Structures and in vitro binding data for the carboline analogs.

Key Research Accomplishments

- completed a structure-activity relationship study of our lead compound and identified properties for critical for assuring a high affinity and selectivity for σ_2 receptors;

- conducted a biodistribution study with a first generation ^{18}F-labeled compound in tumor-bearing rodents. Although the data were encouraging, giving a suitable tumor:background ratio for imaging purposes at 4 hr post-i.v. injection (Figures 1 and 2), we believe it will be necessary to prepare a compound having a higher affinity for σ_2 receptors in order achieve a suitable tumor-background ratio at an earlier time point;
- confirmed that the rigid-analog approach as described in the original grant application will lead to σ_2 selective compounds.

Reportable Outcomes.

None at this point in time. However, we anticipate having two manuscripts submitted for publication within the next three months.

Conclusion.

The results of the research conducted during the first year of this IDEA award suggest that it will be possible to prepare a ^{18}F-labeled imaging agent for assessing the σ_2 receptor status of breast tumors. The initial compound that was evaluated in vivo, [^{18}F]**1b**, produced a suitable tumor:background ratio for imaging purposes, but required 4 hr post-i.v. injection to reach this value. The goal of the next year is to prepare a ^{18}F-labeled analog having a high affinity for σ_2 receptors than [^{18}F]**1b** so that a high tumor:background ratio can be achieved in a shorted time point, which should be better suited for conducting imaging studies in breast cancer patients.

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